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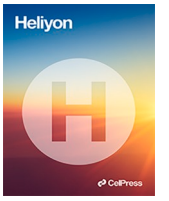

Research article

Temporary alleviation of MAPK by arbutin alleviates oxidative damage in the retina and ARPE-19 cells

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ABSTRACT

Dry age-related macular degeneration (AMD) is one of the main diseases that causes blindness in humans, and the number of cases is increasing yearly. However, effective treatments are unavailable, and arbutin (ARB) has been reported to have antioxidant, anti-inflammatory, and anti-aging effects in other age-related diseases. However, whether ARB can be used to treat dry AMD remains unknown. To explore the therapeutic potential and molecular mechanism of arbutin in the treatment of dry AMD. MTT assays, reactive oxygen species (ROS) production assays, flow cytometry assays, qPCR and western blotting were used to assess the impact of ARB on human RPECs induced by H_2O_2. A transcriptome sequencing assay was used to further explore how ARB acts on human RPECs treated with H_2O_2. Hematoxylin and eosin (H&E) staining and total antioxidant capacity (T-AOC) assays were used to observe the impact of ARB on mouse retina induced by sodium iodate. ARB counteracted the H_2O_2-induced reduction in human RPECs viability, ARB reversed H_2O_2-induced cellular ROS production by increasing the expression of antioxidant-related genes and proteins, ARB also reversed H_2O_2-induced cell apoptosis by altering the expression of apoptosis-related genes and proteins. Transcriptome sequencing and western blotting showed that ARB reduced ERK1/2 and P-38 phosphorylation to prevent H_2O_2-induced oxidation damage. The in vivo experiments demonstrated that ARB protected against retinal morphology injury in mice, increased serum T-AOC levels and increased antioxidant oxidase gene expression levels in the mouse retina induced by sodium iodate. We concluded that ARB reversed the H_2O_2-induced decrease in human RPECs viability through the inhibition of ROS production and apoptosis. The ERK1/2 and P38 MAPK signaling pathways may mediate this process. ARB maintained retinal morphology, increased serum T-AOC level and improved the expression of antioxidant oxidase genes in mice.

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1. Introduction

Age-related macular degeneration (AMD) is one of the main diseases that causes blindness in humans. The incidence of AMD gradually increases with increasing age. Research studies estimate that the number of patients with AMD worldwide will reach 288 million by 2040 [1]. AMD can be divided into neovascular and nonneovascular AMD; the latter is also called dry AMD or nonexudative AMD and accounts for approximately 85–90 % of the total AMD cases [2]. At present, wet AMD is often treated with retinal photocoagulation or anti-vascular endothelial growth factor therapy, while dry AMD lacks effective treatment. The causes of dry AMD are complex and include smoking, a high-fat diet and oxidative stress, among which oxidative damage to retinal pigment epithelial cells (RPECs) is the most important link [3]. RPECs have a variety of extremely important functions in the retina; for example, they participate in phagocytosis of photoreceptor outer segments (POSs), the transport of metabolic waste and nutrients and oxidative stress [4]. However, RPECs are also easily damaged by excessive reactive oxygen species (ROS). The retina, as the tissue with the highest oxygen consumption per unit weight in the body [5], produces ROS during light stimulation and phagocytosis of POSs, and excessive ROS are harmful to cell survival [6,7]. With increasing age, excessive ROS accumulation leads to oxidative damage to RPECs, damage to organelles including mitochondria, an abnormal POS system, abnormal protein accumulation, RPEC apoptosis, and drusen formation [8], which further impair the visual field. If the pathology continues to develop to an advanced stage, an increase in retinal drusen and map-like atrophy of the retina occur, causing irreversible damage to vision. In general, oxidative stress in human RPECs leads to apoptosis, which is an important link in the pathological process of AMD [9,10], so finding safe and effective antioxidants to treat dry AMD has become a popular research objective, Previous studies have shown that sodium iodate treatment can cause oxidative damage to retina [11], changes in retinal morphology and the formation of drusen-like deposits in mice [12], and SI treatment has been widely used in C57BL/6 mice to establish dry AMD models [13,14].

Arbutin (ARB) is abundant in the leaves of several plants, such as wheat, pear and bearberry [15]. Its effects include antioxidant, anti-inflammatory and antiaging effects. As a common antioxidant, ARB was initially used for skin beautification. Subsequent studies revealed that ARB could delay the progression of age-related degenerative diseases such as Alzheimer's disease and Parkinson's disease and that it could also be used in other tissues, such as Liver and heart. R Wang et al. and Sivasangari et al. respectively described the hepatoprotective efficacy of arbutin against ethanol-induced liver injury and the protective effect of ARB against myocardial infarction in rats [16–18]. and in cells such as optic glial cells and bone cells [19,20]. The strong antioxidant effect of ARB is related to its strong pharmacological effects. For example, ARB can exert its cytoprotective effect by scavenging oxygen free radicals in hepatocytes [21],

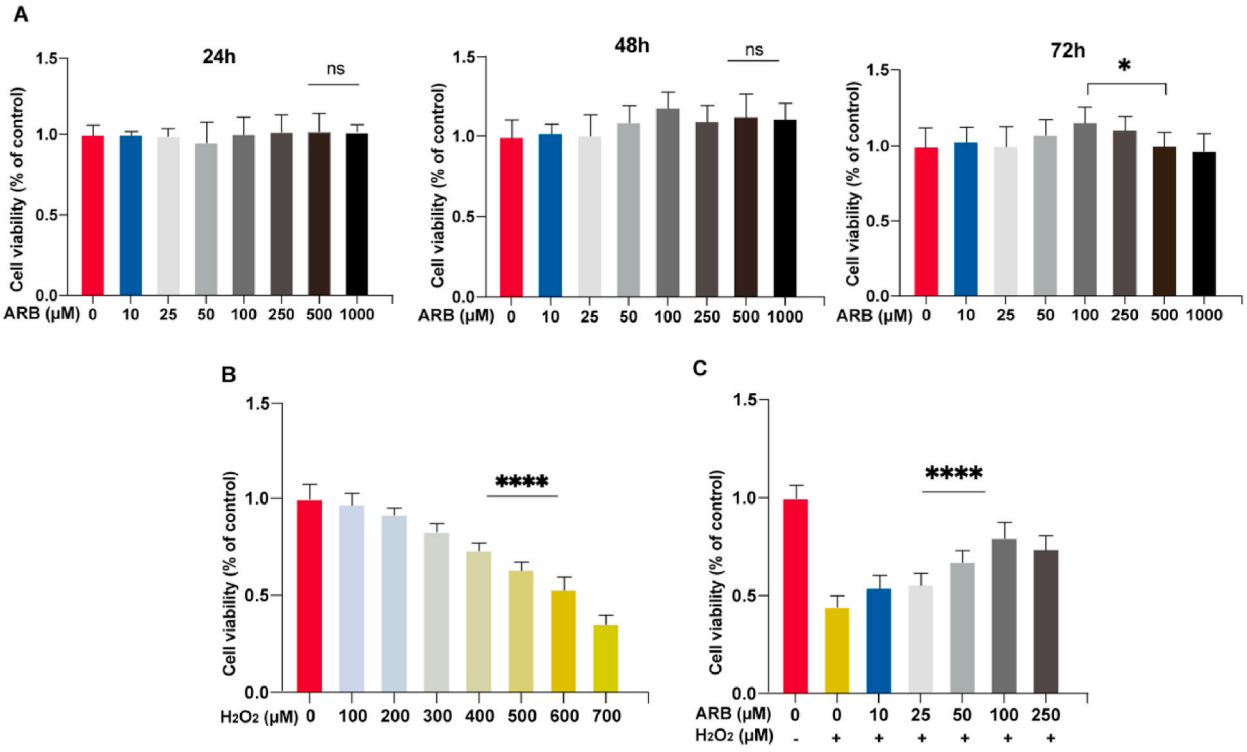

Fig. 1. ARB reverses the decrease in human RPEC viability induced by H_2O_2. A: MTT assays were used to detect the viability of human RPECs treated with 0–1000 μM ARB for 24, 48 and 72 h. B: MTT assays were used to detect the viability of human RPECs treated with different concentrations of 0–700 μM H_2O_2 for 24 h. C: MTT assays were used to detect the viability of human RPECs treated with 600 μM H_2O_2 with or without ARB. One-way ANOVA was used for comparisons among multiple groups, and Tukey's test was used for comparisons between two groups. (($^{ns}P>0.05$, $^{*}P < 0.05$, $^{****}P < 0.0001$).

decrease ROS production in prostate cancer cells for promising complementary medicine in treating prostate cancer [22] and reduce the expression of apoptosis-related factors in human lymphoma cells [23]. ARB is also used in eye diseases. Zhao et al. reported that ARB can alleviate optic nerve injury by attenuating H_2O_2-induced oxidative injury in retinal ganglion cells [19]. However, whether it can help treat dry AMD remains unknown.

In this study, we explored whether ARB could protect RPECs from oxidative damage induced by H_2O_2 and reduce damage in a dry AMD mouse model.

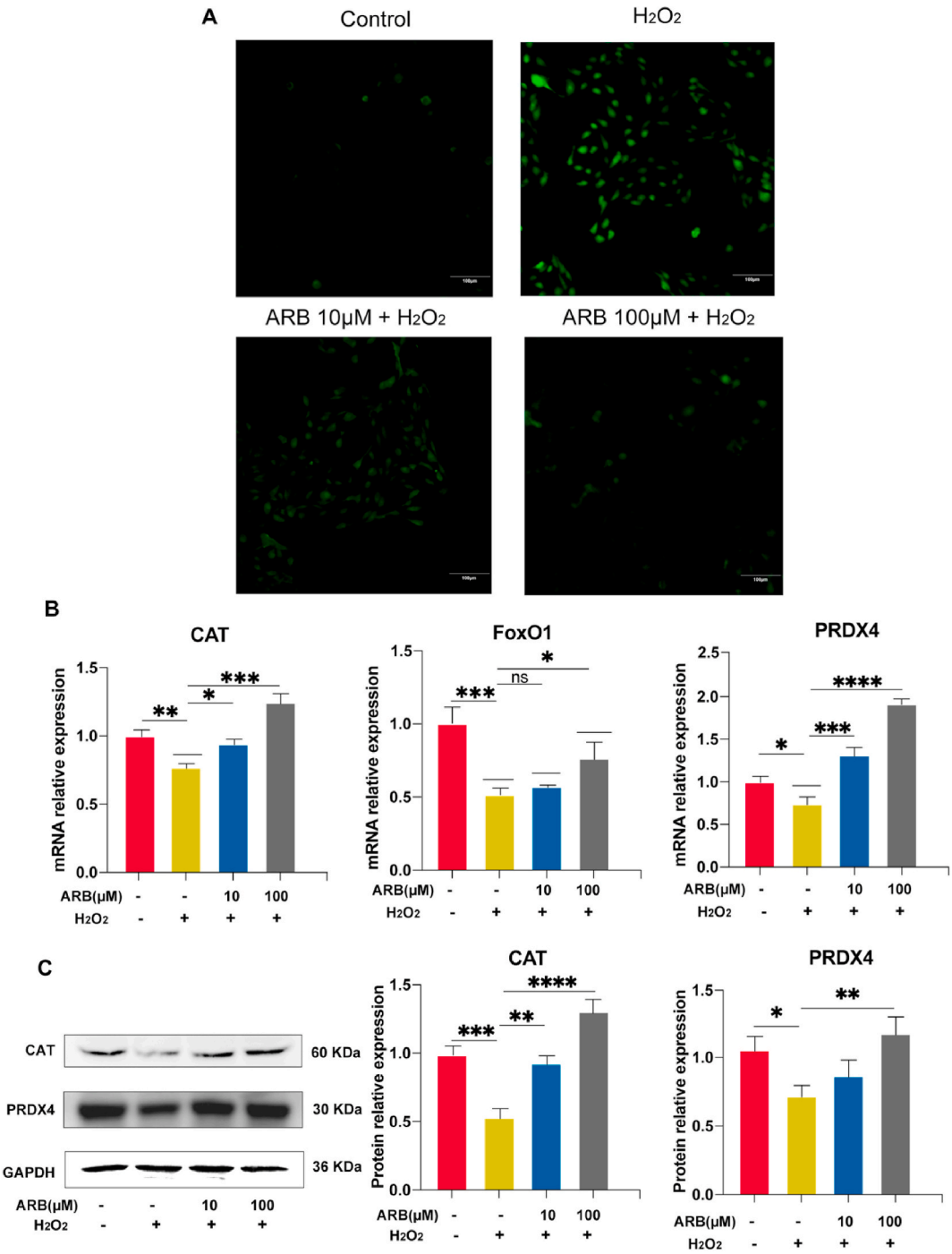

Fig. 2. ARB decreases H_2O_2-induced ROS production by increasing the levels of antioxidant enzymes. A: ROS production was measured by ROS assay (scale bar = 100 μm). B: qPCR assay was used to measure CAT, FoxO1, and PRDX4 gene expression. C: Western blot assay was used to measure CAT and PRDX4 protein expression. ($^{ns}P>0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).

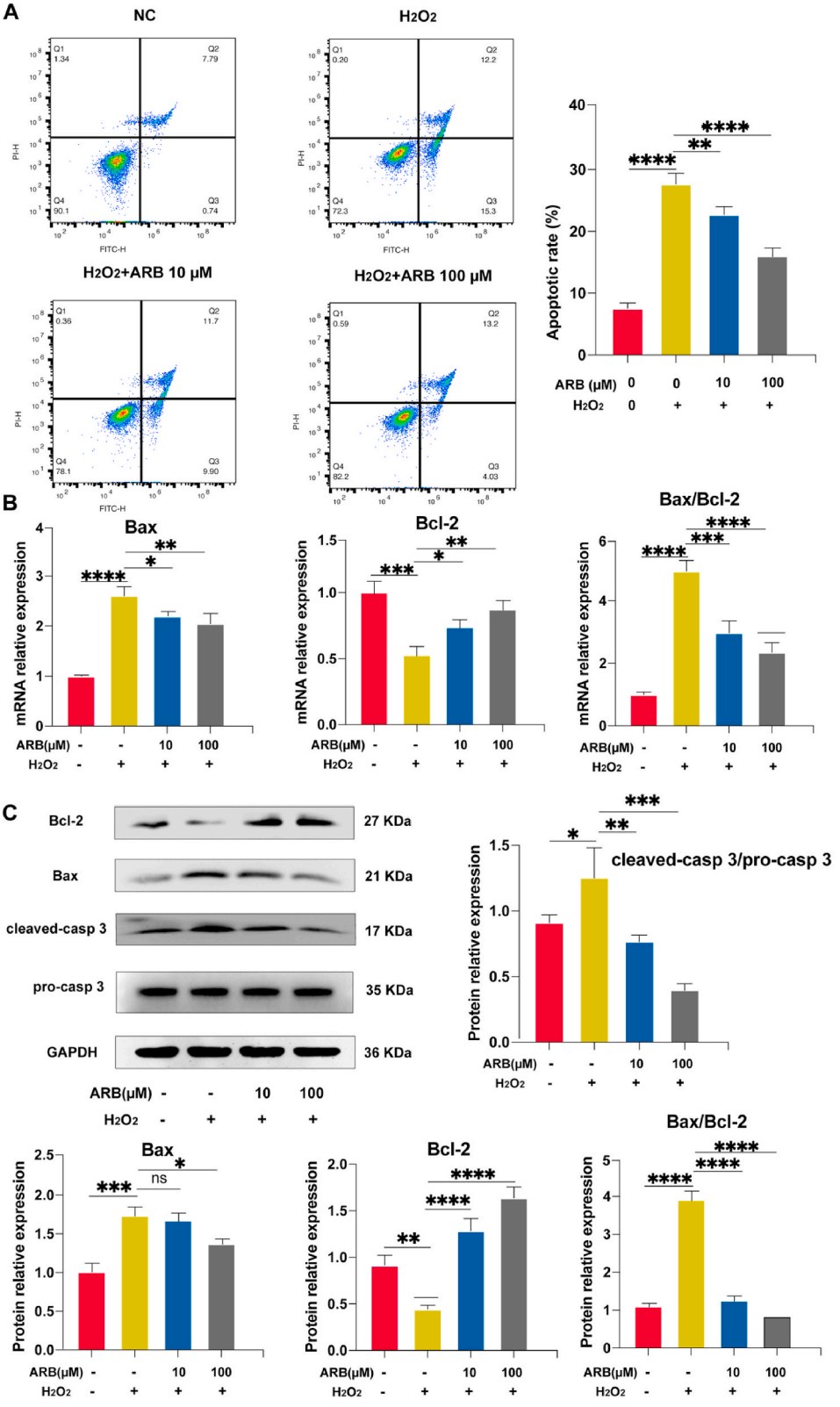

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Fig. 3. ARB reduces H_2O_2-induced apoptosis in human RPECs. A: The apoptosis rate of each group was assessed by flow cytometry (apoptosis rate = Q2+Q3). B: The expression of the apoptosis-related genes Bax and Bcl-2 was measured by qPCR. C: The expression of the apoptosis-related proteins Bax, Bcl-2, cleaved-caspase 3 and pro-caspase 3 was measured by Western blot assay. (^{ns}P>0.05, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

2. Results

2.1. ARB reverses the decrease in human RPECs viability induced by H_2O_2

MTT assay results showed treatment with 0–1000 μM ARB for 24, 48 and 72 h had no toxic effect on human RPECs and human RPECs viability began to decrease when the concentration of ARB was more than 250 μM for 72 h (Fig. 1A). 0–250 μM ARB was used for the next experiment. Moreover, we observed treatment with 600 μM H_2O_2 for 24 h reduced human RPECs viabilty (approximately 50 %) (Fig. 1B), human RPECs stimulated by 600 μM H_2O_2 were used as a model of oxidative damage. We found that the ARB-pretreated group had greater cell viability than the H_2O_2-only group, indicating that ARB reversed the decrease in human RPECs viability induced by H_2O_2. However, the effect of 250 μM ARB was weaker than that of 100 μM ARB (Fig. 1C), therefore, in the next experiment, 10 μM and 100 μM ARB were used in low- and high-concentration groups to observe the effect of ARB on H_2O_2-induced oxidative damage in human RPECs.

2.2. ARB decreases H_2O_2-induced ROS production by increasing the levels of antioxidant enzymes

ROS assays showed that ARB pretreatment inhibited the production of ROS in human RPECs compared to that observed with H_2O_2 treatment alone in a concentration-dependent manner (Fig. 2A). qPCR revealed that compared with H_2O_2 pretreatment alone, ARB pretreatment increased PRDX4, CAT and FoxO1 expression levels when cells pre-treated with ARB at 100 μM, while ARB at 10 μM didn't give any significant result on Fox01 expression (Fig. 2B). Western blot analysis revealed that ARB pretreatment increased CAT and PRDX4 protein expression (Fig. 2C). Overall, ARB alleviated H_2O_2-induced oxidative stress by increasing antioxidant enzyme levels and ROS production in human RPECs.

2.3. ARB reduces apoptosis rate of human RPECs induced by H_2O_2

The Annexin V-PI flow cytometry assay showed that the apoptosis rate in the ARB-pretreated group was decreased in a concentration-dependent manner compared with that in the H_2O_2-only group (Fig. 3A). qPCR showed that at the gene level, the expression of Bax was lower, the expression of Bcl-2 was higher, and the ratio of Bax to Bcl-2 was lower in the ARB pretreatment group than in the H_2O_2 treatment group (Fig. 3B). The changes in Bax and Bcl-2 were also confirmed at the protein level. Furthermore, we observed that there was no significant change in the level of pro-casp-3 protein in the ARB-pretreated group, but the expression of cleaved-casp-3 protein was decreased significantly (Fig. 3C). These findings suggest that ARB pretreatment can reduce the H_2O_2-induced expression of apoptosis-related genes and proteins in human RPECs.

2.4. ARB alleviates H_2O_2-induced oxidative damage in human RPECs through the MAPK signaling pathway

Transcriptome sequencing revealed a significant difference in gene expression between the H_2O_2-only group and the ARB + H_2O_2 cotreatment group. A differential gene clustering heatmap was used to visualize gene expression in the two groups, which revealed similarities between the individual replicates in each group and significant gene expression differences between the H_2O_2-only group and the ARB + H_2O_2 cotreatment group (Fig. 4A). Together, the sequencing results and KEGG pathway enrichment analysis showed that the ARB-mediated protection against H_2O_2-induced oxidative damage in RPECs may relate to the MAPK signaling pathway (Fig. 4B). Previous studies have also shown that the MAPK signaling pathway plays an important mediating role in the development of dry AMD [24]. Thus, we next measured the expression of proteins related to the MAPK signaling pathway using Western blot analysis. The results showed that the ratio of P–P38 to P38 and the ratio of P-ERK1/2 to ERK1/2 were higher in the H_2O_2 treatment group than in the normal group; however, these changes were attenuated in the ARB pretreatment group compared with the H_2O_2 treatment group (Fig. 4C). Which showed ARB alleviates H_2O_2-induced MAPK pathway activation in human RPECs. To confirm this result, the MAPK signaling pathway inhibitors FR 180204 (ERK MAPK inhibitor) and SB SB203580 (p38 MAPK Inhibitor) were used, and MTT assay results demonstrated that inhibitors effectively reversed the decline in human RPECs viability induced by H_2O_2 (Fig. 4D). Above all, MAPK signaling may have mediated this process.

2.5. ARB protected the retinal morphology of mice and improved the expression of the CAT and SOD2 genes induced by SI

An illustration of dry AMD modeling, intervention and experiment evaluation in mice is showed in Fig. 5A. H&E staining was used to observe retinal morphology and thickness. Retinal drusen-like deposits, the total retinal thickness, inner nuclear layer (INL) thickness, and outer nuclear layer (ONL) thickness were decreased in SI-induced mice, whereas these changes were mitigated by the additional administration of ARB(Fig. 5B). The serum T-AOC detection assay result showed that the ARB-treated group had a greater T-AOC than the group treated with SI alone (Fig. 5C). qPCR was used to further explore the effect of ARBs on the expression of

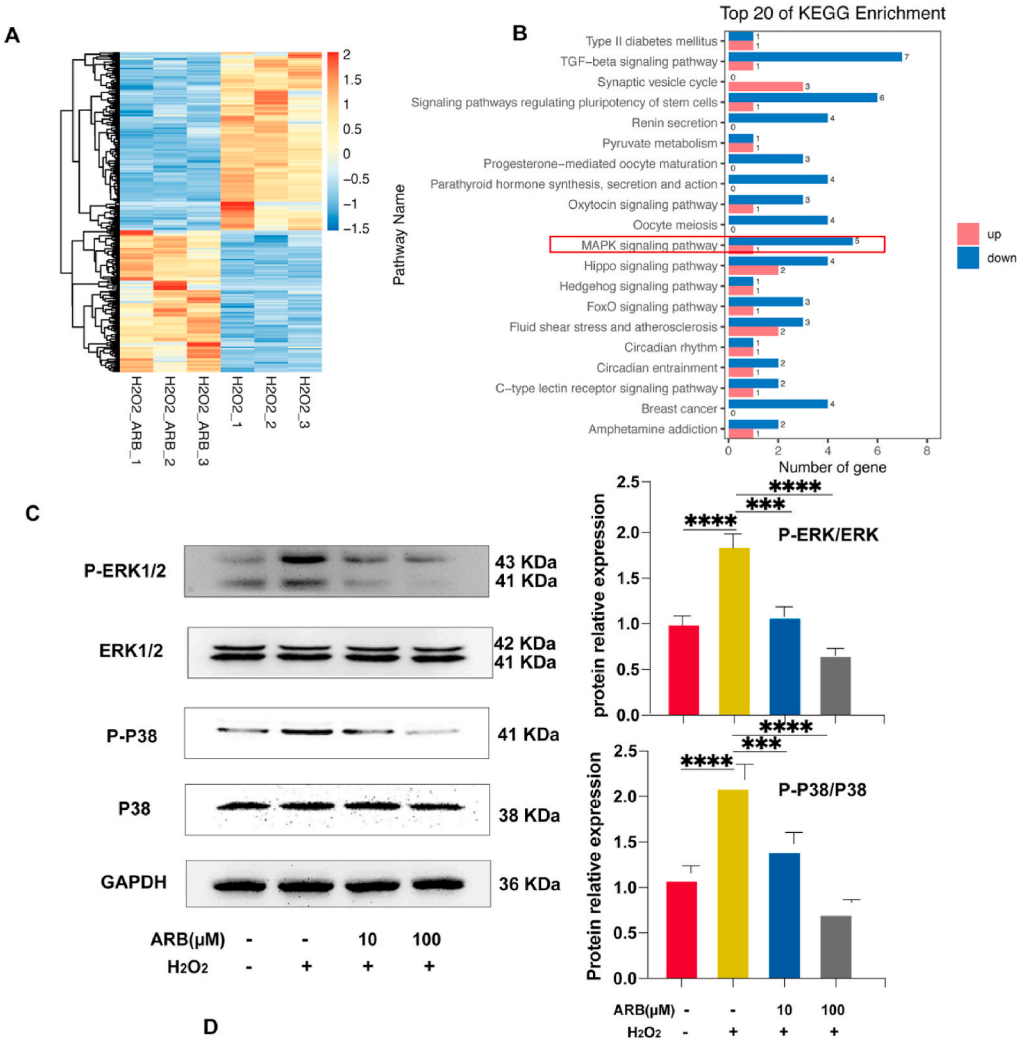

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Fig. 4. ARB alleviates H_2O_2-induced oxidative damage in Human RPECs through the ERK1/2 and P38 MAPK signaling pathways. A: A differential gene clustering heatmap was used to visualize gene expression between the ARB + H_2O_2 group and the H_2O_2 group. B: Transcriptome sequencing results were combined with the KEGG database for pathway enrichment analysis. C: Western blotting was used to measure the expression of the ERK1/2, P-ERK1/2, P38 and P–P38 proteins in each group. D: MTT assays were used to detect the viability of human RPECs pretreated with FR 180204 or SB203580 for 1 h, treated with or without ARB for 24 h, and finally stimulated with H2O2 for an additional 24 h (**P < 0.01, ***P < 0.001, ****P < 0.0001).

antioxidant genes in the mouse retina induced by SI. The results showed that compared with SI treatment alone, ARB treatment increased CAT and SOD2 gene expression levels (Fig. 5D). They are important genes for retinal antioxidant enzymes and risk genes for dry AMD [25], and their increased expression represents an increased ability of the retina to resist oxidative damage [26].

3. Discussion

Our results show that at the cellular level, ARB reversed the H_2O_2-induced decline in human RPEC viability by reducing intracellular ROS levels and apoptosis. MAPK signaling may have mediated this process. Similarly, in vivo experiments demonstrated that ARB maintained retinal morphology in mice with dry AMD, increased serum T-AOC level and improved the expression of antioxidant oxidase genes in mice. In conclusion, ARB is promising for the treatment of dry AMD.

In dry AMD, the retina is characterized by drusen deposition, macular pigment disorder and map-like retinal atrophy. The patient's visual field is damaged in the early stage, and the visual damage is serious and irreversible in the late stage due to severe damage to the macular area. However, there is no effective treatment for dry AMD. The treatments being researched are divided into surgical treatments and drug treatments. The main surgical treatment is stem cell transplantation. However, its safety and ethics and the transplantation technology need to be further optimized [27]. Drug therapy focuses on antioxidation and anti-inflammation. Considering the decisive role of oxidative damage in RPECs in the development of dry AMD, increasing numbers of researchers are working to find antioxidant drugs that inhibit oxidative damage in Human RPECs and prevent the occurrence and development of AMD. At present, the only definite finding regarding dry AMD treatment is that taking vitamin C, vitamin E, beta-carotene, zinc and copper can delay the development of advanced AMD [25]. Some scientists are focusing on natural antioxidant plants.

Previous studies have shown that ARB has antioxidant, antiaging, anti-inflammatory and neuroprotective effects [17]. In vivo experiments have shown that ARB can scavenge free radicals in mouse liver tissue and alleviate acute liver injury [16]. In addition, in vitro experiments have shown that ARB has good oxidation- and apoptosis-inhibiting effects on human retinal glial cells [19]. In this experiment, we explored whether ARB can be used as a potential drug to treat dry AMD at the cellular and animal levels.

Oxidative damage to RPECs is an important factor in the occurrence and development of dry AMD [25], and H_2O_2-induced oxidative damage in RPE cells is a commonly used cell model for dry AMD studies [13,28,29]. This model was used in this study to observe the inhibitory potential and molecular mechanism of ARB on RPE oxidative damage. We observed that ARB reversed H_2O_2-induced RPEC viability decline, which was related to changes in ROS production and the expression of antioxidant enzymes. We found that the increase in RPEC viability induced by H_2O_2 in the 250 μM ARB treatment group was weaker than that in the 100 μM ARB treatment group, which may be related to the increasing cytopathic effects and receptor saturation [30]. This finding requires further exploration. With increasing age, the expression of antioxidant enzymes in the retina gradually declines. CAT catalyzes the decomposition of H_2O_2 into water and oxygen and reduces oxidative stress in the retina. Studies have shown that CAT levels in RPECs in the macula and its surrounding area gradually decrease with age [31]. In vivo and in vitro studies have found that CAT overexpression can not only protect against oxidative damage to the RPE itself induced by H_2O_2 but also reduce the photodamage of surrounding photoreceptor cells [32]. In addition, inhibition of CAT expression during oxidative stress can increase the levels of byproducts of lipid peroxidation in RPECs by 66 % [33]. Our study revealed that the expression of the CAT gene and protein in the ARB pretreatment group was higher than that in the H_2O_2-only group. Moreover, ARB pretreatment increased the gene expression levels of PRDX4 and FoxO1 when cells pre-treated with ARB at 100 μM, while ARB at 10 μM didn't give any significant result on FoxO1 expression, which help to clear ROS from cells and prevent oxidative damage. FoxO1 can protect against oxidative stress by increasing the levels of antioxidant enzymes [34]. PRDX4 is an antioxidant enzyme whose overexpression can improve the antioxidant capacity of cells and inhibit oxidative damage induced by H_2O_2 [35,36].

Apoptosis of RPECs leads to functional damage and decreases the phagocytosis function of photoreceptor cells, which leads to the accumulation of abnormal substances and the formation of drusen. On the other hand, apoptosis of RPECs also directly increases the production of drusen [10]. In the flow cytometry experiment, we observed that ARB inhibited the apoptosis of RPECs induced by H_2O_2; we also observed that ARB changed the expression of apoptosis-related genes and proteins. Previous studies have shown that oxidative stress-induced apoptosis and mitochondrial dysfunction in human RPECs are related to an increase in Bax expression and a decrease in Bcl-2 expression [37,38]. Bcl-2 is a key antiapoptotic member of the Bcl-2 family, which mainly regulates the intrinsic apoptosis pathway [38]. We observed that ARB pretreatment upregulated the expression of Bcl-2 and downregulated the expression of the Bax gene and protein in RPECs treated with H_2O_2. Pro-caspase 3 is the precursor of the apoptotic protein caspase 3, and cleaved caspase 3 is the activated form of caspase 3. In this experiment, we found that ARB inhibited the activation of caspase 3 in a concentration-dependent manner. All these data show that ARB effectively protects human RPECs from H_2O_2-induced oxidative stress through an antiapoptotic effect.

To explore the signaling pathway through which ARB protects against H_2O_2-induced oxidative stress, we examined the sequencing results and performed KEGG signaling pathway analysis. The results showed that the antioxidant effect of ARB is strongly related to the

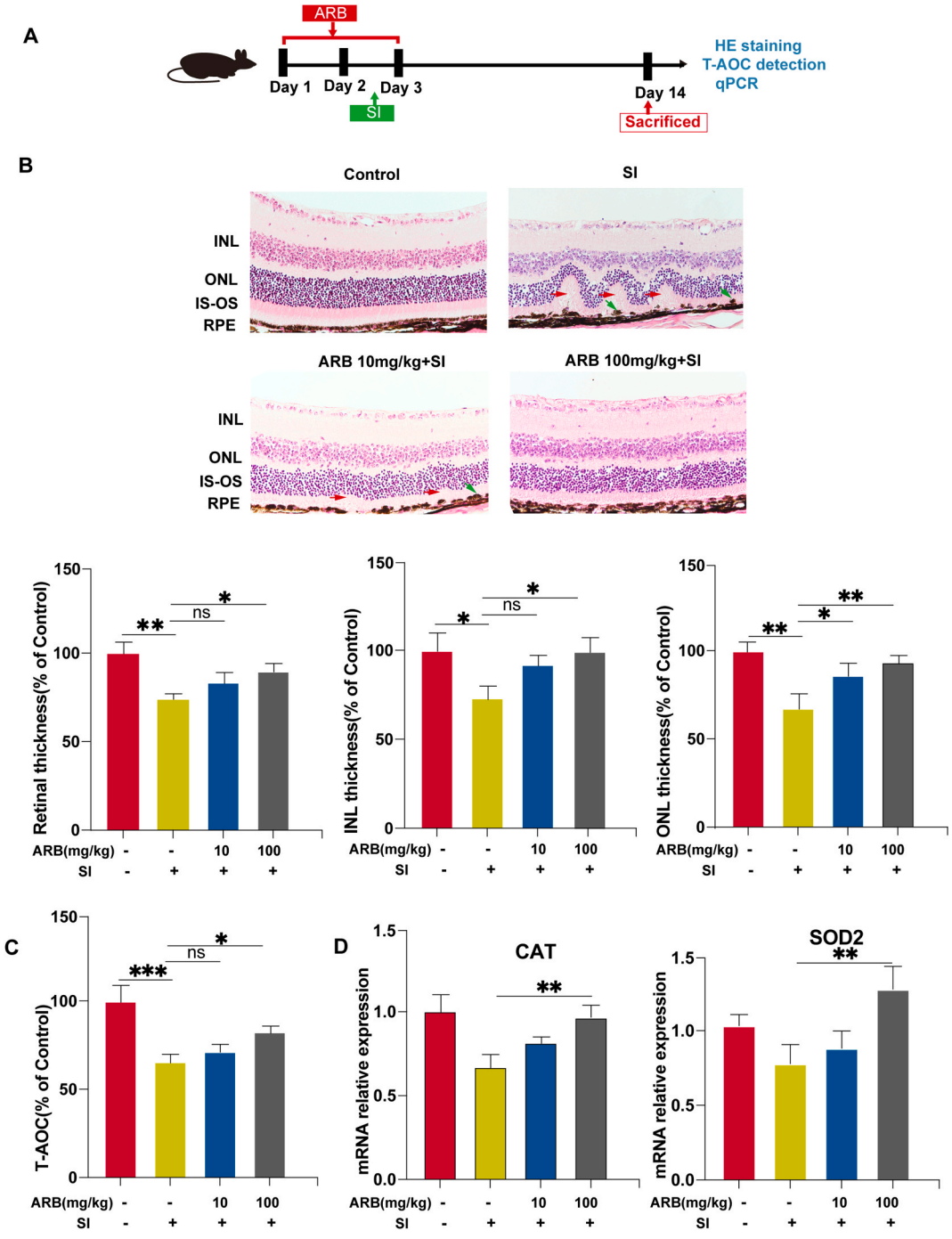

Fig. 5. ARB protects retinal morphology from SI-induced injury in mice. A: Schematic diagram of the experimental design in mice. B: H&E staining was used to observe retinal morphology, retinal thickness, INL thickness and ONL thickness. C: T-AOC results for mouse serum from each group (red arrow and green arrow: drusen-like change). D: qPCR assay was used to measure CAT and SOD2 gene expression. ($^{ns}P>0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

MAPK signaling pathway. The MAPK signaling pathway is an important pathway that interprets extracellular signals; regulates the expression of related genes and proteins; and participates in apoptosis, differentiation, and proliferation. MAPK plays an important role in resistance to oxidative stress and dry AMD in retinal cells, and activation of the ERK1/2 signaling pathway is related to map atrophy in the late stage of dry AMD [24]. In addition, it has been reported that oxidative damage to Human RPECs is related to activation of the ERK1/2 pathway [39] and that activation of the ERK1/2 pathway aggravates oxidative damage [40]. Therefore, we further verified

these roles by Western blot experiments. The results showed that the protein expression levels of P-ERK1/2 and P–P38 in the H_2O_2-treated group were increased but that ARB inhibited this change. In other words, ARB inhibited the H_2O_2-induced activation of the ERK1/2 and P38 MAPK signaling pathways in Human RPECs. We also observed that ERK1/2 and P38 inhibitors blocked the cell viability decline induced by H_2O_2, indicating that ARB does exert its effects through the MAPK signaling pathway.

In the future, we hope that arbutin can be used to treat patients with dry AMD and that the molecular mechanism of action of arbutin can provide more diagnostic and treatment options for the treatment of dry AMD. However, this study also has several limitations. Firstly, the human RPECs used in this study are immortalized cell lines. This is related to the difficulty in obtaining human RPE primary cells. However, further validation of ARB's function on human RPE primary cells may be more convincing in the future. Secondly, in vivo study, we administered ARB by intraperitoneal injection. However, ARB treatment using eye drops, oral administration or intravitreal injection needs to be further explored for subsequent application in future clinical treatments.

4. Materials and methods

4.1. Cell culture

Human RPECs were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium/F-12 (DMEM/F-12, Biological Industries, Kibbutz Beit Haemek, Israel) with 20 % fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA) in a cell incubator at 37 °C under an atmosphere with 5 % carbon dioxide. The cells in each group were pretreated with ARB for 24 h and then treated with H_2O_2 for 24 h. Each experimental group and the specific drug concentrations will be explained in detail in the following experimental methods.

4.2. MTT experiment

An MTT assay was used to measure the viability of RPECs in each group with or without drug treatment. A 200 μl cell suspension was added to each well of a 96-well plate at a cell density of 6000/well. Before pretreatment with 10, 25, 50, 100, 250 or 500 μM ARB for 24 h, cells were treated with FR 180204 (ERK MAPK inhibitor, Beyotime, Shanghai, China) or SB203580 (p38 MAPK inhibitor, Beyotime) for 1 h, and then they were treated with 600 μM H_2O_2 for 24 h or treated with 100, 200, 300, 400, 500, 600 or 700 μM H_2O_2 alone for 24 h. Next, 30 μl of 5 % MTT solution was added, and the plate was incubated at 37 °C for 2 h. Then, 150 μl of DMSO was added, and the absorbance of each well was measured at 490 nm for comparison.

4.3. ROS detection assay

A ROS assay kit (Solarbio, Beijing, China) was used to measure ROS production in RPECs pretreated with ARB and treated with or without H_2O_2. RPECs were incubated with DMEM/F-12 medium containing 10 μM DCFH for 20 min and then observed and imaged with a fluorescence microscope.

4.4. Flow cytometry

Flow cytometry was used to assess the apoptosis rate of RPECs. After cells were treated with ARB at 10 μM and 100 μM for 24 h and then treated with H_2O_2 at 600 μM for 24 h, an Annexin-FITC/PI apoptosis detection kit (BD Bioscience, San Diego, CA, USA) was used to assess the apoptosis rate of each group. Briefly, the cells were resuspended in PBS at 4 °C and then centrifuged (1000 rpm, 3 min) again. This process was repeated twice, after which 500 μl of 1 × binding solution was added to each tube. Then, 100 μl of cell suspension (cell number $>1*10^5$) and 5 μl each of Annexin-FITC and PI were added to a 1.5 ml sterilized EP tube in turn, and the tube was incubated for 15 min at room temperature (25 °C) in a dark room. Next, 400 μl of 1 × binding liquid was added and mixed well. The mixture was filtered and sterilized with a sieve and then placed into a flow tube for analysis. The experimental results of flow cytometry were analyzed with FlowJo software. Apoptosis was calculated as follows: Apoptosis = early apoptosis (Q3) + late apoptosis (Q2).

4.5. qPCR assay

The changes in gene expression levels in each group were measured by qPCR. The mice were sacrificed 14 days after treated with ARB and SI. The eyes were then enucleated from the euthanized mice, and the retinas were excised and dissected from the surrounding tissues. cells were treated with ARB at 10 μM and 100 μM for 24 h and then treated with H_2O_2 at 600 μM for 24 h an RNA extraction kit (EZBioscience, Roseville, USA) was used to extract RNA. After measuring the RNA concentration, RNA, oligo1l, and ddH_2O were added for reverse transcription into cDNA. The cDNA was amplified in a PCR system with a qPCR Kit (TransGen Biotech, Beijing, China). GAPDH and β-actin werec respectively used as the internal reference in human RPECs and mouse retina, and the relative expression of each group was calculated and the relative gene expression value was calculated using the 2ˆ-ΔΔCt method. Each primer designed was listed in Supplementary Table 1.

4.6. Western blot assay

Western blot assays were used to measure the changes in protein expression levels in each group. RIPA buffer was added to extract protein, and the protein concentration was determined by the BCA method. A 5 % concentration gel and an 11 % separation gel were prepared according to the instructions of an SDS–PAGE gel kit. Then, 30 μg of protein was added to each well for electrophoresis. A PVDF membrane was activated with methanol, and the proteins were transferred with a constant current of 250 A for 120 min. After electrophoresis, the membrane was washed with TBST and blocked with 5 % skim milk for 1 h. The membrane was then washed with TBST 3 times and incubated with primary antibodies overnight at 4 °C (anti-catalase, anti-cleaved Caspase-3, anti-pro-Caspase-3, anti-Bax, anti-Bcl-2, anti-ERK, anti-P-ERK, anti-P-38 and anti-p-P38 were purchased from Abcam (Cambridge, MA, USA), PRDX 4 was purchased from proteintech (Hubei, China). Then, the membrane was washed three times with TBST and incubated for 1 h with anti-rabbit and anti-mouse secondary antibodies. The bands were visualized using the chemiluminescence method in a gel image analysis system and analyzed with ImageJ (NIH, Bethesda, MD, USA). Each Western blot image represents at least three independent results.

4.7. RNA extraction and library construction

Cells were pretreated for 24 h with or without 100 μM ARB and then treated with 600 μM H_2O_2 for 24 h. TRIzol was added to extract total RNA. Transcriptome sequencing was performed by Lianchuan Biology (Zhejiang, China). Poly(A) RNA was purified from 1 μg of total RNA. Then, the poly(A) RNA was fragmented using a Magnesium RNA Fragmentation Module (NEB, cat. e6150, USA). The cleaved RNA fragments were reverse-transcribed to create cDNA, which was used to synthesize a U-labeled second-strand of cDNA. The ligated products were amplified with PCR. The average insert size for the final cDNA library was 300 ± 50 bp. Finally, we performed 2 × 150 bp paired-end sequencing (PE150) on an Illumina NovaSeq™ 6000 (LC-Biotechnology CO., Ltd., Hangzhou, China). We used HISAT2 (https://ccb.jhu.edu/software/hisat2) to map the reads to the *Homo sapiens* reference genome GRCh38. StringTie was used to calculate the expression levels of mRNAs by calculating the fragments per kilobase of transcript per million mapped reads (FPKM) values (FPKM = [total_exon_fragments/mapped_reads(millions) × exon_length(kB)]). The differentially expressed mRNAs were selected as those with a fold change >2 or fold change <0.5 and a p value from a parametric F test comparing nested linear models <0.05 using the R package edgeR (https://bioconductor.org/packages/release/bioc/html/edgeR.html).

4.8. Animal experiments

C57/BL6 female mice (6–8 weeks old) were purchased from Weitong Lihua (Beijing, China). The animal procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The mice were fed at 37 °C. After acclimation for one week, the mice were injected intraperitoneally with ARB (10 mg/kg and 100 mg/kg). After an interval of 24 h, the injection of ARB was repeated. Sodium iodate (SI) at 65 mg/kg was injected into the tail vein 12 h later, and then ARB was injected intraperitoneally 12 h later. The mice were euthanized after 14 days.

4.9. Hematoxylin and eosin (H&E) staining

The eyeballs were fixed with eyeball fixation solution for 48 h, dehydrated in an automatic dehydrator, embedded in paraffin, sliced with a 0.2 μm slicer, baked at 63 °C for 2 h, and stained with H&E.

4.10. Total antioxidant capacity (T-AOC) assay

The animals were treated with ARB and SI for 14 days according to the protocol described above. Orbital blood was collected. The sample was centrifuged at 5000 r/min for 10 min to obtain serum, and a T-AOC assay kit (BC1315, Solarbio, Beijing, China) was used to measure the antioxidant capacity. First, standard solutions with different concentrations were prepared. The absorbance was measured, and the mixture was prepared according to the instructions. Then, 6 μl of sample, 18 μl of ddH2O, and 180 μl of the above mixture were mixed and allowed to react at room temperature for 10 min. The absorbance of each group was measured at 593 nm and compared with the standard curve. The antioxidant capacity was calculated according to the serum volume of each group.

4.11. Data analysis

The data are expressed as the mean ± standard deviation. Prism 9 was used to make charts and analyze the data. ANOVA was used for statistical analysis, and Tukey's test was used to determine the differences between two or more groups.

5. Conclusion

Our results showed that, at the cellular level, ARB reversed H_2O_2-induced decline in human RPE cell viability by reducing intracellular ROS survival and apoptosis, MAPK signaling may mediate this process. Similarly, at the animal level, ARB maintained retinal morphology, increased serum T-AOC level and improved the expression of antioxidant oxidase genes in mice. In conclusion, we concluded that ARB is promising for the treatment of dry AMD.

Ethics approval

This experimental protocol was approved by the Experimental Animal Ethics Committee of Nankai Hospital (NKYY-DWLL-2023-075), in line with ARVO's Statement on the Use of Animals in Ophthalmology and Vision Research.

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Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE265933.

CRediT authorship contribution statement

Ling Wang: Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Ye Tian:** Investigation, Data curation. **Liangpin Li:** Supervision, Resources. **Maoyu Cai:** Visualization, Supervision. **Xueyan Zhou:** Visualization. **Wangming Su:** Software. **Xia Hua:** Resources, Funding acquisition, Conceptualization. **Xiaoyong Yuan:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Xiaoyong Yuan reports was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32887.

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